

## Polyethylene Oxide and their Antibacterial Effects Against some Pathogenic Bacteria

Abtisam Jasim Abbas<sup>1</sup>, Farah Tareq Al-Alaq<sup>2</sup>, Lubna Abdulazee<sup>3</sup>, Ahmed Samir Naje<sup>4\*</sup>

<sup>1</sup> College of Science, AL-Qadisiyah University, Al Diwaniyah, Qadisiyyah Province, Iraq

<sup>2</sup> Department of Biology, College of Science, University of Babylon, Babylon, Iraq

<sup>3</sup> DNA Research Center, Babylon University, Babylon, Iraq

<sup>4</sup> Collage of Water Resource Engineering, AL-Qasim Green University, Babylon, 51031, Iraq

\* Corresponding author's e-mail: [ahmednamesamir@yahoo.com](mailto:ahmednamesamir@yahoo.com)

### ABSTRACT

Poly ethylene oxide is an uncrosslinked, non-ionic linear hydrophilic polymer with a variety of molecular weights. PEO is used to make it, and it offers a number of beneficial qualities for medication delivery and antibacterial uses. The antibacterial activity of polyethylene oxide (PEO) at various concentrations as (80, 40, 20, 10 g/ml) against bacteria in Gram-positive *Staphylococcus aureus*, *Streptococcus pyogenes* and *Lactobacillus* sp. and Gram-negative *Enterobacter bugandensis*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* was investigated in this study. The disk diffusion experiment was used to assess the antimicrobial activity of PEO, as well as each isolate's minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). PEO is shown to have strong broad-spectrum antibacterial action against the bacteria studied, that inhibition zone increase their width inversely proportional to PEO concentration, and has even outpaced the efficacy of certain medicines. PEO had MICs ranging from 10 to 20 g/ml, as well as MBCs of 20 to 80 g/ml. In additional studies, PEO was discovered to be strongly associated with the cell of bacteria, which contributed to its inhibitory impact on bacterial invasion and growth. PEO at an appropriate dose effectively decreased bacterial growth. PEO is highly recommended as a cost-effective antibacterial treatment, Specifically, ectopic infection treatment without the risk of bacterial strains becoming antibiotic-resistant.

**Keywords:** polyethylene oxide, minimum inhibitory concentration, antibacterial activity.

### INTRODUCTION

The growing concern about the rise of more drug-resistant strains of bacteria, viruses and fungi and that endangers people's life and health encourages the rapid development of microbiological safety materials. It is expected that, as a result of the SARS-CoV-2 coronavirus pandemic, there will be a significant increase in the material used as antimicrobial coatings in the next years [1].

Nosocomial infections are caused by bacteria such as *Escherichia coli*, *Streptococcus* and *Staphylococcus* in the United States, 2 million patients are affected each year and result in 90,000 fatalities [2]. Microbial infections are a concern

that impacts more than just hospitals. Many bacteria can produce an exopolysaccharide matrix, a protective matrix of DNA, proteins, and polysaccharides, when they are deposited on item surfaces (EPS). Biofilms are microbial colonies that are encased with EPS. It's 1000 times more difficult to destroy germs after they've formed a biofilm [3]. Synthetic polymer materials are employed in water, ventilation, and other composites systems because they degrade slowly. These are ideal conditions for the long-term colonization of materials by microbes, and they may serve as a breeding ground for harmful germs [4].

Polyethylene oxide (PEO) is a biocompatible, non-toxic and water soluble polymer with

a wide range of applications, including conductive with carbon black, cosmetics (personal lubricants, emulsions, skin creams), medical products and gene therapy [5-10]. Graft copolymers based on PEO have been studied for a number of qualities as well as methods to improve and change their capabilities [8-9]. These materials have been employed in a variety of applications, including lithium batteries, drug delivery systems, elastomer production, nanotechnology [6, 10–14], also biomedical implants [15, 16].

In this study, we try to shed light on the isolation of different pathogenic bacteria in different human location and their susceptibility test patterns, then, the antibacterial activity of PEO were study against the same isolate of pathogenic bacteria to evaluate their capacity.

## MATERIALS AND METHODS

### Bacterial isolates

The bacteria were isolated from patients of mouth infection at Hillah Teaching Hospital. For isolation and purification, all samples were cultured on MacConkey's and blood agar plates at 37 °C for 24–48 hours. Vitek 2 compact system (Biomérieux) confirmed all of the isolates.

### Solution and media

Hi-Media, a Mumbai, India-based company, provided Mueller-Hinton agar and Mueller-Hinton media. Polyethylene oxide (PEO) and dimethyl sulfoxide (DMSO) were provided by Polyethylene Oxide (PEO) and Dimethyl Sulfoxide (DMSO), respectively (Zhengzhou Dongyao Nano Materials Co., Ltd. China). Doxycyclin (DO-30), Ciprofloxacin (KF-30), Clarithromycin (CLR-15), Novobiocin (NV-5) and Methicillin (ME-5) were among the antibiotic disks ordered (Bioanalyse, Turkey).

### Antibacterial activity of PEO

PEO antibacterial activity, was tested against Gram-positive *Lactobacillus* sp., *Staphylococcus aureus* and *Streptococcus pyogenes* as well as Gram-negative *E. coli*, *Enterobacter bugandensis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which were kept on nutritional agar slants. The antibacterial activity was determined using the recommendations of the (CLSI)

Clinical and Laboratory Standards Institute. [17]. A disk diffusion assay is performed to assess antibiotic sensitivity and PEO against the bacteria under research, with triplicates utilized in dilutions of PEO concentrations (80, 40, 20, and 10 µg/ml) by using deionized and sterile water. All isolates initially incubated at room temperature for 15 minutes before being transferred to 37 °C for a period overnight. After a time of incubation, was seeing the inhibition zone around the well recording as a positive results. Using digital Vernier calipers, the diameter of the inhibitory zone was measured [18].

### The determination of a minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [19]

Before being used to create 0.5 McFarland, the bacteria isolates were grown overnight at 37 °C. After generating a total of 10 ml of nutritious broth medium in tubes, all samples was kept in an aseptic environment infected with 1ml of the appropriate bacterial suspension with about (108 CFU/mL). Four PEO dilutions (80, 40, 20, and 10) as well as a negative control were prepared in deionized sterile water (free PEO). All tests were conducted in triplicate for each isolate. Overnight at 37 °C, the infected sets were incubated. After time of incubation, the visual turbidity within every tube was assessed. There is no turbidity at the lowest concentration is considered the MIC for all tested isolate. MIC tubes with no seen turbidity when incubated overnight at 37 °C on nutrient agar plates. The MBC was determined by monitoring the development of bacterial colonies and identifying the concentration that indicated no growth.

## RESULTS AND DISCUSSION:

According to the findings of the present study (100 samples), the most common Gram positive bacteria isolated from human infections were *Streptococcus pneumonia* (10%), *Staphylococcus aureus* (18%), and *Lactobacillus* sp. (12%), in addition to Gram negative bacteria *Escherichia coli* (20%), *Enterobacter bugandensis* (13%), *Klebsiella pneumonia* (10%), and *Pseudomonas aeruginosa* (10%). Table 1 shows the results.

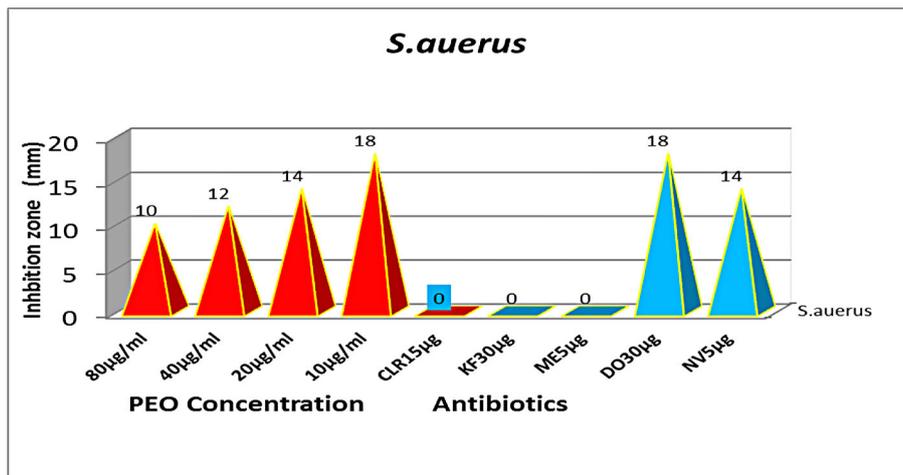
**Table 1.** Bacterial isolates from odontogenic infection, by number and percentage

Bacterial isolates	Total	%
<i>Strep. pneumonia</i>	10	10
<i>Staph. aureus</i>	18	18
<i>E. coli</i>	20	20
<i>Enterococcus faecalis</i>	13	13
<i>Lacto. sp.</i>	12	12
<i>Klebsiella pneumoniae</i>	10	10
<i>Pseudomonas aeruginosa</i>	17	17
Total	100	100

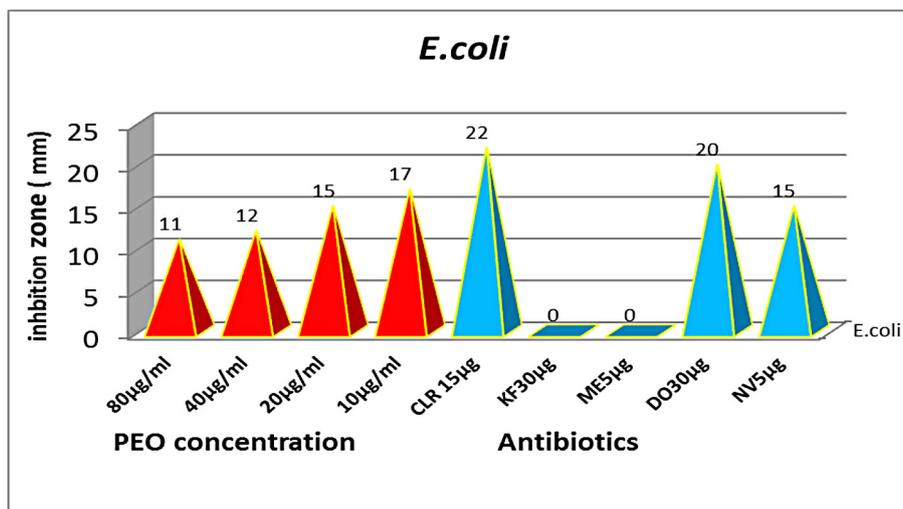
Antibiotic sensitivity testing was performed on each species of bacteria using a modified Kirby-Bauer disc diffusion method. As indicated in the image, Antibiotics that are selective are the most effective widely utilized in bacterial infection to demonstrate their impact on various populations (1-7).

### Antibacterial activity of PEO

PEO demonstrates that multidrug microorganisms are evaluated for potent broad-spectrum antibacterial action. We compared the effects of several antibiotics on pathogenic isolates of bacteria. The Figures 1 to 7 explains that all antibiotic discs used were ineffective against bacterial isolates tested. PEO polymer showed diameter of inhibition zone as clearly directly proportional with the decreased of PEO concentrations that exceeded the effectiveness of several medicines. 10 µg/ml concentration of PEO gives highest inhibition zone area against the bacterial pathogenic isolates, 18 mm appeared maximum zone of PEO inhibition against *Staphylococcus aureus* (Fig. 1) and the least isolate was affected in the sensitive in comparison with the selected antibiotic discs followed by *Streptococcus*



**Figure 1.** Antibacterial activity of PEO on *Staphylococcus aureus*



**Figure 2.** Antibacterial activity of PEO on *E. coli*

*pneumonia* (Fig. 4). Second sensitive bacterial isolate to PEO is *E. coli* as in Figure 2, finally *Enterobacter bugandensis*, *P. aeurogenosa* and *Step. pnumonia* (Fig. 3, 4 and 6).

PEO causes a quick loss of integrity of the bacterial cell membrane, as well as the production of reactive oxygen species (ROS), including superoxide species, which contributes to

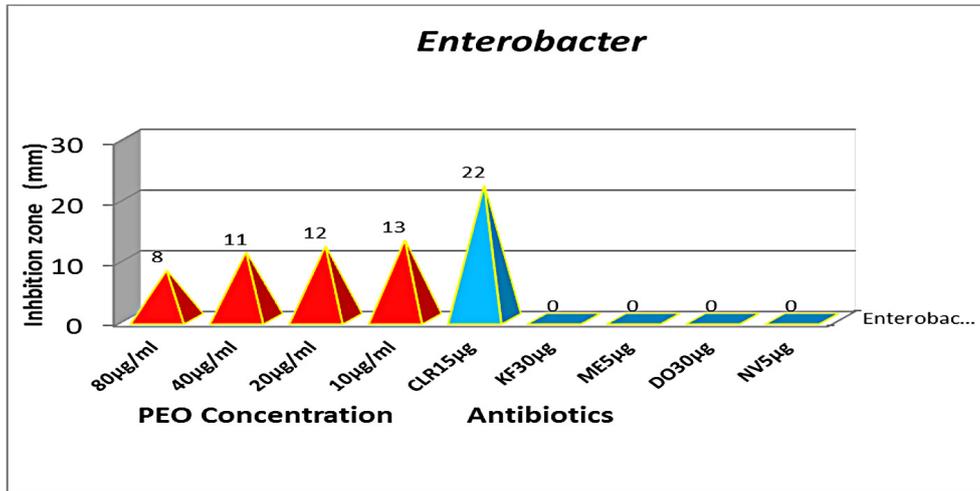


Figure 3. Antibacterial action of PEO on *Enterobacter*

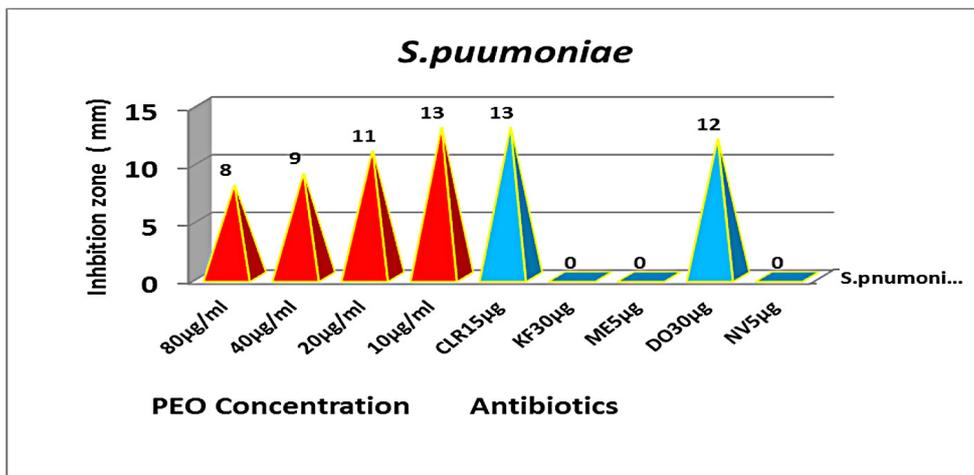


Figure 4. Antibacterial activity of PEO on *Step. pnumoniae*

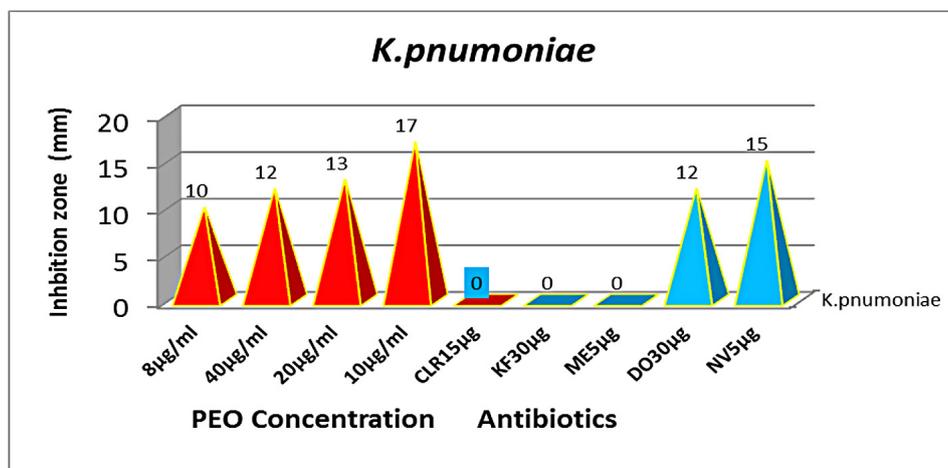


Figure 5. Antibacterial activity of PEO on *K. pnumoniae*

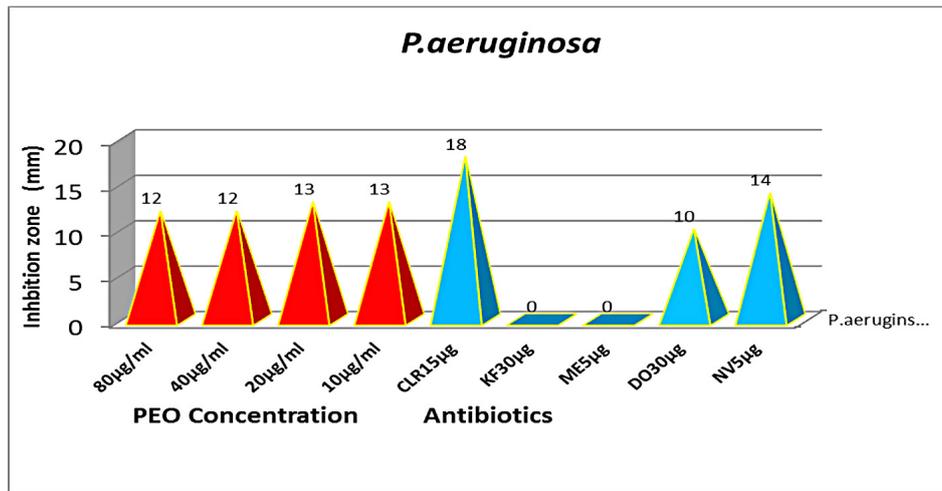


Figure 6. Antibacterial activity of PEO on *P. aeruginosa*

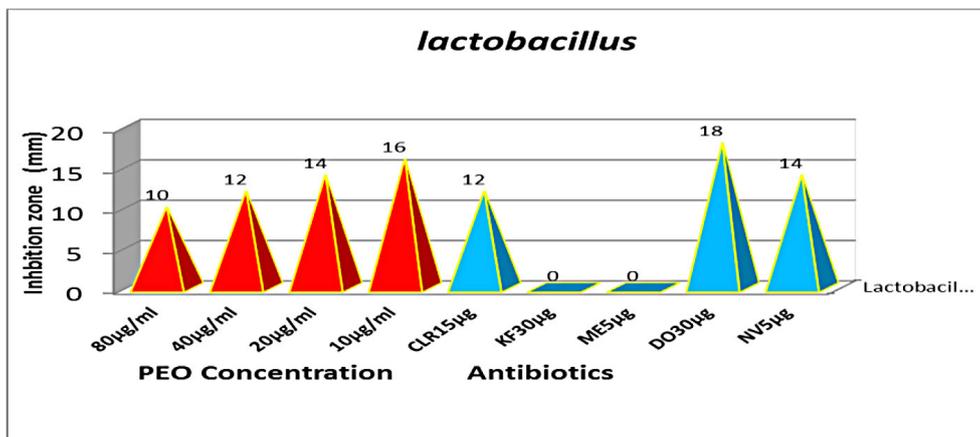


Figure 7. Antibacterial activity of PEO on *Lactobacillus sp.*

biomolecule destruction [20]. Acquisition of non-susceptibility to at least one antibacterial antibiotic or category among three or more antibacterial antibiotics or categories was characterized as minimal residual illness [21]. All results was agreed with other study the Zhang and Chen [22] explain that PEO have ability to inhibited multi-drug-resistant bacteria (MDR).

Table 2. PEO MIC and MBC to pathogenic isolates of bacteria

Bacterial isolates	MIC µg/ml	MBC µg/ml
<i>Strep. pneumonia</i>	10	20
<i>Staph. aureus</i>	20	40
<i>E. coli</i>	20	80
<i>Enterobugandensis</i>	20	40
<i>Lacto. sp.</i>	10	20
<i>Kleb. pneumonia</i>	20	40
<i>Pseudo. aeruginosa</i>	20	80

**The determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)**

Table 2 reveals that the value MIC of PEO was 10 to 20 µg/ml, and the MBC was 20 to 80 µg/ml, with *Staph. aureus* having the highest sensitivity followed by other bacteria.

**CONCLUSION**

According to the findings of this investigation, PEO has a significant inhibitory and antibacterial impact on selected pathogenic isolates of bacteria from the human infected mouth. Because of its efficient capacity to suppress bacterial growth, PEO is highly suggested antibacterial agent as a low-cost substitute, particularly in materials used to create toothpastes, mouthwashes, and dental fillings.

## REFERENCES

- Bates, C.M., Chang, A.B., Momečilović, N., Jones, S.C., Grubbs, R.H. 2015. ABA Triblock Brush Polymers: Synthesis, Self-Assembly, Conductivity, and Rheological Properties. *Macromolecules*, 48, 4967–4973.
- Clinical and Laboratory Standards Institute, CLSI. 2006.
- Clinical and Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational, Supplement. CLSI Document M02-A10 and M07-A8. Texas: Clinical and Laboratory Standards Institute.
- Clinical and Laboratory Standards Institute. 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational, Supplement. CLSI Document M02-A10 and M07-A8. Texas: Clinical and Laboratory Standards Institute.
- CLSI. 2016. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute.
- Gabriel, G.J., Som, A., Madkour, A.E., Eren, T., Tew, G.N. 2007. Infectious disease: Connecting innate immunity to biocidal polymers. *Mater. Sci. Eng. R. Rep.*, 57, 28–64.
- Gao, A.X., Liao, L., Johnson, J.A. 2014. Synthesis of acid-labile PEG and PEG-doxorubicin-conjugate nanoparticles via Brush-First ROMP. *ACS Macro. Lett.* 2014, 3, 854–857.
- Gasteier, P., Reska, A., Shult, P., Salber, J., Offenhäuser, A., Moeller, M., Groll, J. 2007. Surface grafting of PEO-based star-shaped molecules for bioanalytical and biomedical applications. *Macromol. Biosci.*, 7, 1010–1023.
- Gueugnon, F., Denis, I., Pouliquen, D., Collette, F., Delatouche, R., Héroguez, V., Grégoire, M., Bertrand, P., Blanquart, C. 2013. Nanoparticles Produced by Ring-Opening Metathesis Polymerization Using Norbornenyl-poly(ethylene oxide) as a Ligand-Free Generic Platform for Highly Selective In Vivo Tumor Targeting. *Biomacromolecules*. 2013, 14, 2396–2402.
- Johnson, J.A., Lu, Y.Y., Burts, A.O., Xia, Y., Durrell, A.C., Tirrell, D.A., Grubbs, R.H. 2010. Drug-loaded, bivalent-bottle-brush polymers by graft-through ROMP. *Macromolecules* 2010, 43, 10326–10335.
- Kim, S.C., Lee, D.K. 2005. Preparation of TiO<sub>2</sub>-coated hollowglass beads and their application to the control of algal growth in eutrophic water. *Microchem J.*, 80, 227–232.
- Kugel, A., Stafslin, S., Chisholm, B.J. 2011. Antimicrobial coatings produced by “tethering” biocides to the coating matrix: A comprehensive review. *Prog. Org. Coat.*, 72, 222–252.
- Lavery, A.L., Primpke, S., Lorenz, C., Gerdtts, G., Dobbs, F.C. 2020. Bacterial biofilms colonizing plastics in estuarine waters, with an emphasis on *Vibrio* spp. and their antibacterial resistance. *PLoS ONE* 15, e0237704.
- Liao, L., Liu, J., Dreaden, E.C., Morton, S.W., Shopsowitz, K.E., Hammond, P.T., Johnson, J.A. 2014. A convergent synthetic platform for single-nanoparticle combination cancer therapy: Ratiometric loading and controlled release of cisplatin, doxorubicin, and camptothecin. *J. Am. Chem. Soc.*, 136, 5896–5899.
- Liu, J., Burts, A.O., Li, Y., Zhukhovitskiy, A.V., Ottaviani, M.F., Turro, N.J., Johnson, J.A. 2012. ‘Brush-first’ method for the parallel synthesis of photocleavable, nitroxide-labeled poly(ethylene glycol) star polymers. *J. Am. Chem. Soc.*, 134, 16337–16344.
- Neugebauer, D. 2007. Graft copolymers with poly(ethylene oxide) segments. *Polym. Int.* 2007, 56, 1469–1498.
- Pemmada, R., Zhu, X., Dash, M., Zhou, Y., Ramakrishna, S., Peng, X. 2020. Science-based strategies of antiviral coatings with viricidal properties for the COVID-19 like pandemics. *Materials*, 13, 4041.
- Quémener, D., Chemtob, A., Héroguez, V., Gnanou, Y. 2005. Synthesis of latex particles by ring-opening metathesis polymerization. *Polymer*, 46, 1067–1075.
- Radder, A.M., Leenders, H., van Blitterswijk, C.A. 1996. Application of porous PEO/PBT copolymers for bone replacement. *J. Biomed. Mater. Res.*, 30, 341–351.
- Vargas, K.F., Borghetti, R.L., Moure, S.P., Salum, F.G., Cherubini, K., Figueiredo, M.A.Z. 2012. Use of polymethylmethacrylate as permanent filling agent in the jaw, mouth and face regions—implications for dental practice. *Gerodontology*, 29, e16–22. DOI: 10.1111/j.1741-2358.2011.00479.x
- Xue, Z., He, D., Xie, X. 2015. Poly(ethylene oxide)-based electrolytes for lithium-ion batteries. *J. Mater. Chem. A*(3), 19218–19253.
- Zhang, H., Chen, G. 2009. Potent antibacterial activities of Ag/TiO<sub>2</sub> nanocomposites powders synthesized by a one-pot sol-gel method. *Environ Sci Technol.*, 34, 2905–2910.
- Zhou, H., Schön, E.-M., Wang, M., Glassman, M.J., Liu, J., Zhong, M., Díaz, D.D., Olsen, B.D., Johnson, J.A. 2014. Crossover experiments applied to network formation reactions: Improved strategies for counting elastically inactive molecular defects in PEG gels and hyperbranched polymers. *J. Am. Chem. Soc.*, 136, 9464–9470.